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Synthesis and antimicrobial activity of new adamantane derivatives I✪★

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A series of fourteen derivatives of adamantane was synthesised. The new compound 4-(adamant-1-ylmethoxycarbonyl)phthalanhydride obtained from 1-adamantanemethanol and trimellitic anhydride chloride appeared very useful for preparation of a number of N-substituted phthalimides. Antimicrobial activity of the newly obtained derivatives such as, for example, 4-(adamant-1-ylmethoxycarbonyl)-*N*-(5-carboxypentamethylene)phthalimide or 4-(adamant-1-ylmethoxycarbonyl)-*N*-(L-alanyl)phthalimide was tested against *Staphylococcus aureus, Bacillus sp., Micrococcus flavus* and *Enterococcus faecium*. The minimal inhibitory concentration (MIC) for these compounds against *S. aureus* were 0.022 and 0.05 μg/ml, respectively.

Trimellitic acid anhydride (4-carboxyphthalanhydride) (TMAA) is used in many fields of organic chemistry. Some of ester-imides derived from this anhydride, e.g. compounds with an amino acid as N-substituent and a cholesteryl, phenyl or biphenyl moiety-containing ester group, exhibit liquid-crystal properties [1–3]. Trimellitic anhydride is also frequently used as substrate for poly(ester imide)s synthesis. The combination of the rigid and flat phthalimide core with mesogenic properties of the ester structure endows such polymers with a liquid crystalline character [4–8]. Many compounds possessing imide rings in their structure exhibit biological activity. For example, some imides are valuable

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Abbreviations: AM, 1-adamantanemethanol; AU, *N*-(adamant-1-yl)urea; DMF, dimethylformamide; Me_2SO, dimethylsulfoxide; MIC, minimal inhibitory concentration; TMAA, trimellitic acid anhydride; TMAC, trimellitic anhydride chloride; TNF, tumor necrosis factor.

substrates for the production of anti-seizure drugs [9]. The most known of the clinically useful imides is the antiviral drug — amantidine (1-aminoadamantane) [10]. Another field where amantidine and many related derivatives are successfully employed, is the treatment of certain neurological disorders, e.g. Parkinson's disease [11]. There are only few papers concerning the antimicrobial activity of adamantane derivatives [12–14]. In this study we show that certain easily synthesise 1-adamantanemethanol esters of various N-substituted phthalimide 4-carboxylates exhibit a distinct antimicrobial activity. It is worth to note that among structurally similar compounds *N*-(1-adamantyl)maleimide shows anticancer activity in mice and inhibits herpes simplex virus replication *in vitro* [14, 15]. *N*-Adamantylphthalimide induces tumor necrosis factor (TNF-α) enhancing activity induced by 12-*O*-tetradecanoylphorbol-13-acetate in human leukaemia HL-60 cells [16]. We expect that a combination of both types of compounds, those with a flat phthalimide core and those with a three-dimensional "box-like" adamantane structure, will provide a broad range of active compounds for biological and pharmacological investigations.

MATERIALS AND METHODS

General methods

Melting points were taken in open capillary tubes on a Gallenkamp 5 melting point apparatus and were uncorrected. The structures of products were confirmed by elemental analysis, FTIR and ^{1}H NMR spectroscopy. The NMR spectra were measured on a Varian Gemini 200 MHz spectrometer in $CDCl_3$ or d_6-Me_2SO solutions. Column flash chromatography was performed on silica gel 60 (Merck). FTIR spectra were recorded on a Perkin Elmer 2000 apparatus using the KBr pellet method. Adamantane and phthalic acid derivatives were purchased from Aldrich. The specific rotation of compounds was determinated on a Perkin Elmer polarimeter at 20°C in chloroform (c =1 g/100 cm^3) using the D line of sodium. Preliminary testing of the antimicrobial activity of the newly synthesised compounds was performed by the disc diffusion method using Mueller-Hinton agar medium under standard conditions as described by NCCLS [17]. Sterile filter paper discs were soaked in test compounds solutions prepared in EtOH-Me_2SO mixture (v/v, 1:1). The results were read following 18 h incubation at 37°C (for *M. flavus* at 30°C). Compounds showing distinct antimicrobial activity in the above test were next tested for MIC (minimal inhibitory concentration) in liquid Mueller-Hinton medium according to the appropriate NCCLS protocol, using original stock solutions [18]. *Staphylococcus aureus* NCTC 4163 and *Enterococcus faecium* ATCC 6057 were purchased from the National Institute of Hygiene (Warsaw, Poland) *Bacillus subtilis* H17rec$^-$ and *Bacillus subtilis* M45rec$^+$ were kindly donated by dr. T. Kada from the National Institute of Genetics (Misima, Japan) the other microorganisms used were from own collection of the Department of Pharmaceutical Microbiology, Medical University (Warsaw, Poland).

Syntheses

N-(Adamant-1-yl)trimellitimide **(1)**: 1.92 g (10 mmol) of trimellitic anhydride and 2.28 g (15 mmol) of 1-aminoadamantane were dissolved in dry DMF (15 cm^3) and refluxed for 5 h. Then the mixture was cooled and poured into aqueous 1% HCl. Crude product was filtered and crystallised from 90% ethanol to give **1** (1.17 g, 52%); m.p. 261°C; FTIR (cm^{-1}): C=O$_{imide}$ 1706 and 1768. ^{1}H NMR ($CDCl_3$) δ(ppm): 1.75–2.52 (3m, H$_{adamantane}$), 7.85–8.45 (m, H$_{arom.}$). Elemental analysis: calculated for $C_{19}H_{19}NO_4$: C, 70.14; H, 5.89; N, 4.30. Found: C, 70.55; H, 5.95; N, 4.23.

N-(Adamant-2-yl)trimellitimide **(2)**: Compound **2** was synthesised analogously to the procedure described above for **1** starting from

2-aminoadamantane and trimellitic anhydride in a yield 0.90 g (40%); m.p. 228°C; FTIR (cm^{-1}): $C{=}O_{imide}$ 1714 and 1771. ^1H NMR ($CDCl_3$) δ(ppm): 1.79–2.92 (3m, $H_{adamantane}$), 7.85–8.49 (m, $H_{arom.}$). Elemental analysis: calculated for $C_{19}H_{19}NO_4$: C, 70.14; H, 5.89; N, 4.30. Found: C, 70.50; H, 5.89; N, 4.22.

4-(N^1-(Adamant-1-yl)ure-N^2-ylcarbonyl))phthalic acid **(3)**: 0.420 g (2 mmol) of trimellitanhydride chloride and 0.39 g (2 mmol) of *N*-(adamant-1-yl)urea were dissolved in anhydrous pyridine and the reaction mixture was stirred overnight at room temperature. A pale yellow solution was evaporated to an oil and the residue was coevaporated with water to remove the rest of pyridine. Twice crystallisation from methanol water gave **3** (345 mg, 45%) m.p. 205°C; FTIR (cm^{-1}): $C{=}O_{amide}$ 1680, $C{=}O_{acid}$ 1695, $C{=}O_{urea}$ 1728. ^1H NMR (d_6-Me_2SO) δ(ppm): 1.62–2.62 (3 m, $H_{adamantane}$), 7.65–8.22 (m, $H_{arom.}$). Elemental analysis: calculated for $C_{20}H_{22}N_2H_6$: C, 62.17; H, 5.74; N, 7.25. Found: C, 62.10; H, 5.54; N, 7.41.

4-(Adamant-1-yl-methoxycarbonyl)phthalanhydride **(4)**: The synthesis of **4** was performed from 1-adamantanemethanol and trimellitic anhydride chloride according to the method described previously [19, 20]. Crude product was crystallised with benzene. Yield 42%; m.p. 158°C; FTIR (cm^{-1}): $C{=}O_{ester}$ 1731, $C{=}O_{anh.}$ 1785 and 1857.

4-(Adamant-1-ylmethoxycarbonyl)phthalic acid **(5)**: 1.70 g (5 mmol) of **4** was heated with water for 15 min. After cooling the crystals precipitated. Yield 1.43 g (40%); m.p. 197°C. FTIR (cm^{-1}): $C{=}O_{acid}$ 1702, $C{=}O_{ester}$ 1732. ^1H NMR ($CDCl_3$) δ(ppm): 1.65–2.04 (m, $H_{adamantane}$), 3.99 (s, CH_2O), 7.82–8.54 (m, $H_{arom.}$). Elemental analysis: calculated for $C_{20}H_{22}O_6$: C, 67.03; H, 6.19. Found: C, 67.10; H, 6.29.

N-substituted-4-(adamant-1-ylmethoxycarbonyl)phthalimides **(6–12)**: 1.36 g (5 mmol) of **4** and appropriate amino acids were refluxed in dry DMF for 3 h. Then mixtures were poured into diluted HCl (1%). Crude products were purified by column chromatography (silica gel) using chloroform/methanol/acetic acid (v/v, 25 : 1 : traces) as an eluent.

4-(Adamant-1-ylmethoxycarbonyl)-N-carboxymethylenephthalimide **(6)**: (m.p. 242°C, 1.03 g, 65%); FTIR (cm^{-1}): $C{=}O_{imide}$ 1722 and 1773; ^1H NMR ($CDCl_3$) δ(ppm): 1.63–2.02 (m, $H_{adamantane}$), 3.99 (s, CH_2O), 4.52 (s, N–CH_2), 7.90–8.48 (m, $H_{arom.}$). Elemental analysis: calculated for $C_{22}H_{23}NO_6$: C, 66.49; H, 5.83; N, 3.52. Found: C, 66.44; H, 5.89; N, 3.44

4-(Adamant-1-ylmethoxycarbonyl)-N-(2-carboxydimethylene)phthalimide **(7)**: (m.p. 162°C, 0.99 g, 60%); FTIR (cm^{-1}): $C{=}O_{imide}$ 1723 and 1778; ^1H NMR ($CDCl_3$) δ(ppm): 1.62–2.02 (m, $H_{adamantane}$), 2.80 (t, $CH_2C{=}O$), 3.97 (s, CH_2O), 4.02 (t, N–CH_2), 7.93–8.44 (m, $H_{arom.}$). Elemental analysis: calculated for $C_{23}H_{25}NO_6$: C, 67.14; H, 6.12; N, 3.40. Found: C, 67.25; H, 6.02; N, 3.52.

4-(Adamant-1-ylmethoxycarbonyl)-N-(3-carboxytrimethylene)phthalimide **(8)**: (m.p. 161°C, 0.95 g, 56%); FTIR (cm^{-1}): $C{=}O_{imide}$ 1718 and 1779; ^1H NMR ($CDCl_3$) δ(ppm): 1.63– 1.73 (m, $H_{adamantane}$), 1.63–1.65 [m, $(CH_2)_3$], 2.03 (m, $-CH_2-$), 2.43 (t, $CH_2C{=}O$), 3.79 (t, N–CH_2), 3.98 (s, CH_2CO), 7.95–8.45 (m, $H_{arom.}$). Elemental analysis: calculated for $C_{24}H_{27}NO_6$: C, 67.75; H, 6.40; N, 3.29. Found: C, 67.34; H, 6.49; N, 3.33.

4-(Adamant-1-ylmethoxycarbonyl)-N-(5-carboxypentamethylene)phthalimide **(9)**: (m.p. 168°C, 1.23 g, 68%); FTIR (cm^{-1}): $C{=}O_{imide}$ 1718 and 1779; ^1H NMR ($CDCl_3$) δ(ppm): 1.63–2.02 (m, $H_{adamantane}$), 3.99 (s, CH_2O), 4.52 (s, NCH_2), 7.90–8.48 (m, $H_{arom.}$). Elemental analysis: calculated for $C_{26}H_{31}NO_6$: C, 68.86; H, 6.89; N, 3.09. Found: C, 68.76; H, 6.66; N, 2.99.

4-(Adamant-1-ylmethoxycarbonyl)-N-(L-alanyl)phthalimide **(10L)**: (m.p. 117°C, 0.69 g, 42%); FTIR (cm^{-1}): $C{=}O_{imide}$ 1725 and 1780; ^1H NMR ($CDCl_3$) δ(ppm): 1.62–2.02 (m, $H_{adamantane}$ and CH_3), 3.97 (s, CH_2O), 4.98 (q, N–CH), 7.90–8.44 (m, $H_{arom.}$); $[\alpha]_D^{20} = -7.6°$. Elemental analysis: calculated for: $C_{23}H_{25}$-

NO_6 : C, 67.14; H, 6.12; N, 3.40. Found: C, 67.28; H, 6.01; N, 3.50.

4-(Adamant-1-ylmethoxycarbonyl)-N-(D-alanyl)phthalimide **(10D)**: (m.p. 118°C, 0.68 g 40%); FTIR (cm^{-1}): C = O_{imide} 1724 and 1780; ^1H NMR ($CDCl_3$) δ(ppm): 1.62–2.02 (m, $H_{adamantane}$ and CH_3), 3.97 (s, CH_2O), 4.98 (q, N–CH), 7.90–8.44 (m, $H_{arom.}$); $[\alpha]_D^{20}$= +7.6°. Elemental analysis: calculated for: $C_{23}H_{25}NO_6$: C, 67.14; H, 6.12; N, 3.40. Found: C, 67.27; H, 6.00; N, 3.45.

4-(Adamant-1-ylmethoxycarbonyl)-N-(L-phenylalanyl)phthalimide **(11L)**: (m.p. 115°C, 1.07 g, 55%); FTIR (cm^{-1}): C=O_{imide} 1725 and 1780; ^1H NMR ($CDCl_3$) δ(ppm): 1.61–2.02 (m, $H_{adamantane}$), 3.54 (m, CH_2–phenyl), 3.94 (s, CH_2O), 5.08 (m, N–CH), 7.16 (s, $H_{phenylala.}$), 7.37–8.34 (m, $H_{arom.}$); $[\alpha]_D^{20}$= –94.5°. Elemental analysis: calculated for: $C_{29}H_{29}NO_6$: C, 71.44; H, 6.00; N, 2.87. Found: C, 72.00; H, 6.03; N, 2.99.

4-(Adamant-1-ylmethoxycarbonyl)-N-(D-phenylalanylo)phthalimide **(11D)**: (m.p. 115°C, 1.11 g, 57%); FTIR (cm^{-1}): C=O_{imide} 1725 and 1781; ^1H NMR ($CDCl_3$) δ(ppm): 1.61–2.02 (m, $H_{adamantane}$), 3.54 (m, CH_2–phenyl), 3.94 (s, CH_2O), 5.08 (m, N–CH), 7.17 (s, $H_{phenylala.}$), 7.37–8.35 (m, $H_{arom.}$); $[\alpha]_D^{20}$= +94.3°. Elemental analysis: calculated for: $C_{29}H_{29}NO_6$: C, 71.44; H, 6.00; N, 2.87. Found: C, 71.96; H, 6.03; N, 2.95.

4-(Adamant-1-ylmethoxycarbonyl)-N-(4-carboxyphenyl)phthalimide **(12)**: (m.p. 289°C, 1.38 g, 75%); FTIR (cm^{-1}): C = O_{imide} 1708 and 1775; ^1H NMR ($CDCl_3$) δ(ppm): 1.64–2.02 (m, $H_{adamantane}$), 4.02 (s, CH_2O), 7.37–8.60 (m, $H_{arom.}$). Elemental analysis: calculated for: $C_{27}H_{25}NO_6$: C, 70.58; H, 5.48; N, 3.05. Found: C, 71.01; H, 5.50; N, 3.11.

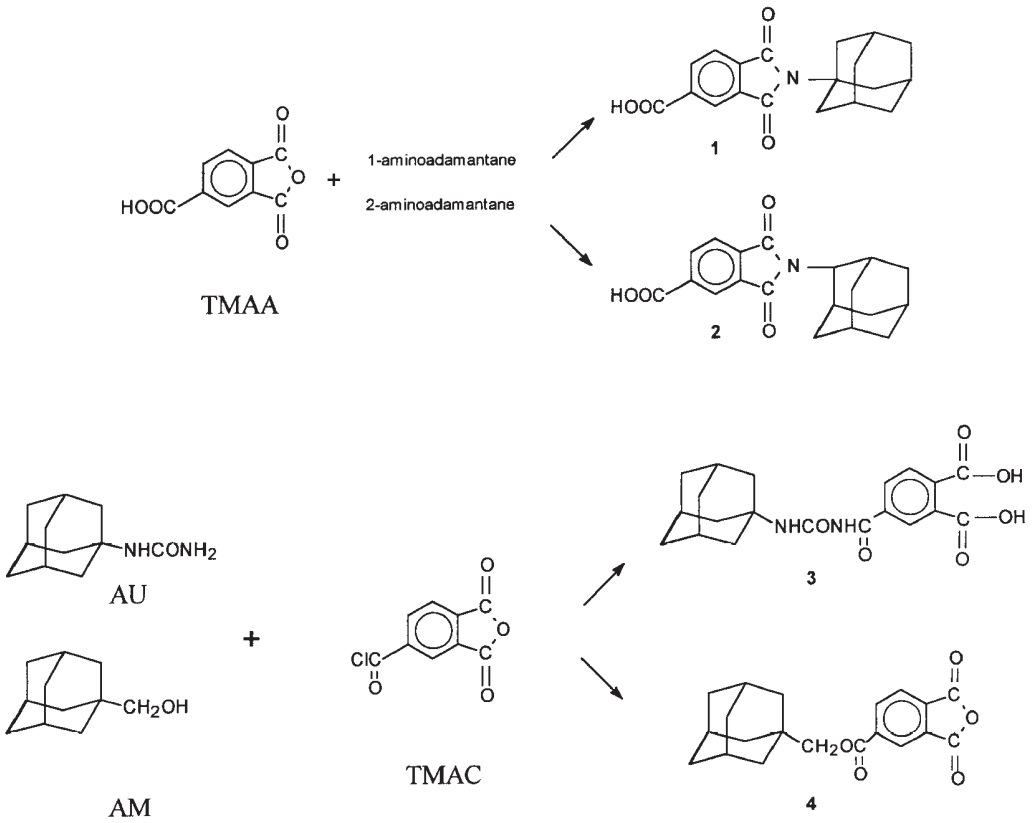
RESULTS AND DISCUSSION

Chemical synthesis

Highly reactive trimellitic acid anhydride (TMAA) (Scheme 1) was utilised for the synthesis of N-substituted phthalimide derivatives in the reaction with 1-aminoadamantane and 2-aminoadamantane. The reaction was performed in refluxing anhydrous DMF to give, respectively: *N*-(adamant-1-yl)trimellitimide **1** and *N*-(adamant-2-yl)trimellitimide **2**. Stirring of commercially available trimellitic anhydride chloride (TMAC) with *N*-(adamant-1-yl)urea (AU) in pyridine at ambient temperature followed by hydrolytic opening of the anhydride yielded 4-(N^1-(adamant-1-yl)urea-N^2-ylcarbonyl))phthalic acid **3**. Esterification of TMAC with 1-adamantanemethanol (AM) analogously to the method described earlier for cholesteryl derivate synthesis [1–3, 19, 20] yielded 4-(adamant-1-ylmethoxycarbonyl)phthalanhydride **4**. Thermally facilitated hydrolysis of **4** in water gave 4-(adamant-1-ylmethoxycarbonyl)phthalic acid **5** (Scheme 2). Heating **4** with ω-aminomethylenecarboxylic acids, D- and L-alanine and enantiomers of phenylalanine, as well *p*-aminobenzoic acid analogously to the aforementioned procedure resulted in N-substituted trimellitimides **6**–**12**, respectively.

Microbiological studies

The antimicrobial activity of adamantyl derivatives of phthalimide was first tested by the agar disc-diffusion method against Gram-positive bacteria: *Staphylococus aureus, Micrococcus flavus, Enterococcus faecium* and certain strains of *Bacillus*. Gram-negative bacteria: *Bordella bronchiseptica, Pseudomonas aeruginosa* and strains belong to the family *Enterobacteriaceae* as well as the fungus *Candida albicans* were resistant to all tested compounds. Next, the minimal inhibition concentration (MIC) of the most active compounds was determined in liquid Mueller-Hinton medium (Table 1). A particularly strong antimicrobial activity, comparable to that of clinically used antibiotics, was observed for N-amino-acid substituted derivatives **10** and **11**. Both chiral isomers, i.e. **10L**, **10D**, and **11L**, **11D** were highly active



Scheme 1.

against *Micrococcus flavus* and *Staphylococcus* strains. In contrast, **11L** and **11D** were practically inactive against *Enterococcus faecium,* whereas **10L** and **10D** showed moderate activity against this microorganism. The most distinct differences in the antimicrobial activ-

Table 1. Sensitivity of Gram-positive *cocci* strains to certain adamantyl derivatives of substituted phthalimides

Bacteria strain	Minimal inhibitory concentration (MIC) in μg/ml											
	Compound											
	1	**2**	**3****	**6**	**7**	**8**	**9**	**10L**	**10D**	**11L**	**11D**	**12**
S. aureus ATCC 25923	7.5	7.5	10	7.5	5.0	5.0	0.022	0.22	0.22	0.05	0.05	na
S. aureus ATCC 6538P	7.5	7.5	10	7.5	5.0	5.0	0.08	0.22	0.22	0.015	0.05	na
S. aureus NCTC 4163	7.5	7.5	10	7.5	5.0	5.0	0.08	0.22	0.22	0.05	0.05	na
S. aureus 31	7.5	7.5	na	7.5	5.0	2.5	0.08	0.15	0.15	0.05	0.05	15.0
M. flavus NCIB 8166	2.5	3.5	na	10.0	2.5	2.5	0.8	0.55	0.55	0.022	0.8	na
E. faecium ATCC 6057	na*	na	na	na	na	5–7.5	na	7.5	7.5	na	na	na

*na – no activity at concentration > 50 μg/ml; **compounds **4** and **5** were inactive against bacteria strains tested.

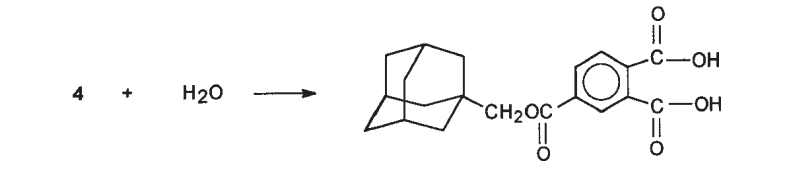
Table 2a. Sensitivity of *Bacillus* strains to adamantyl derivatives of substituted phthalimide

Bacteria strain	Diameter of growth inhibition area (mm)												
	Compound *												
	1	**2**	**3****	**6**	**7**	**8**	**9**	**10L**	**10D**	**11L**	**11D**	**12**	**Control**
B. subtilis ATCC 6633	14	13	0	17	18	11	12	29	43	39	35	10	16[a]
B. cereus ML 98	15	14	10	18	20	17	15	33	25	33	37	10	14[a]
B. stearothermophilus ATCC 7953	15	14	10	18	20	18	15	40	28	33	33	10	14[a]
B. subtilis M45 rec$^-$	14	14	10	19	19	17	15	45	33	33	33	10	28[b]
B. subtilis H17 rec$^+$	14	14	10	19	19	19	15	39	39	29	33	10	12[b]

*800 μg per 8 mm disc; **compounds **4** and **5** were inactive; [a]nitrofurantoine 300 μg per 8 mm disc; [b]4-nitrochinoline *N*-oxide 1 μg per 8 mm disc

ity of both chiral isomers **11L** and **11D** were observed for *S. aureus* ATCC 6538P and *M. flavus* strains.

Noteworthy are large differences in the antimicrobial activity obtained by the disc-diffusion method and MIC method with *Bacillus*



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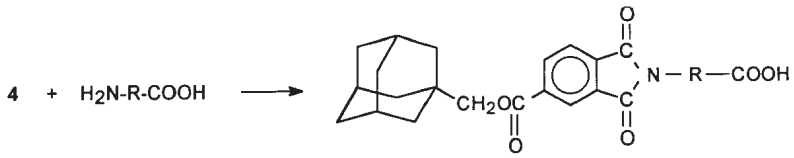

6-12

compound	R	compound	R
6	$-CH_2-$	10Land 10D	$-\dot{C}H-CH_3$
7	$-(CH_2)_2-$	11Land 11D	$-\dot{C}HCH_2-C_6H_5$
8	$-(CH_2)_3-$	12	$-C_6H_5$
9	$-(CH_2)_5-$		

Scheme 2

Table 2b. Sensitivity of *Bacillus* strains to adamantyl derivatives of substituted phthalimides

Bacteria strain	Minimal inhibitory concentration (MIC) in μg/ml		
	Compound		
	1-9, 10L and D, 12	**11L**	**11D**
B. subtilis ATCC 6633	>50.0	37.5	30.0
B. cereus ML 98	>50.0	35.0	30.0
B. stearothermophilus ATCC 7953	>50.0	30.0	30.0
B. subtilis M45 rec$^-$	>50.0	18.5	35.0
B. sublitis H17 rec$^+$	>50.0	35.0	35.0

strains (Tables 2a and 2b). A similar discrepancy was previously observed for these aerobic bacteria in antimicrobial tests of essential oil from *Tanacetum partenium* [21]. The high sensitivity of *Bacillus* strains may be useful in testing the antimicrobial activity of next adamantane derivatives, the synthesis of which we plan in the nearest future.

The genetically modified *Bacillus* rec$^+$ and rec$^-$ strains employed in the present study were previously shown to be useful in screening the genotoxicity of antibiotics under development [22]. Results obtained in the course of the present study (details not shown) showed no genotoxicity of the newly synthesised adamantane antimicrobials. We plan to expand these studies to include other N-aminoacyl-substituted phthalimidecarboxylic acid esters of adamantane.

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